Respiratory Culture Organism Isolation and Test Characteristics in Children with Tracheostomies with and without Acute Respiratory Infection

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Abstract

Background: Among children with tracheostomies, little is known about how respiratory culture results differ between states with and without acute respiratory infections (ARI), or the overall test performance of respiratory cultures. **Objective**: To determine the association of respiratory culture organism isolation with diagnosis of ARI in children with tracheostomies, and assess test characteristics of respiratory cultures in the diagnosis of bacterial ARI (bARI). **Methods**: This single-center, retrospective cohort study included respiratory cultures of children with tracheostomies obtained between 2010-2018. The primary predictor was ARI diagnosis code at the time of culture; the primary outcomes were respiratory culture organism isolation and species identified. Generalized estimating equations were used to assess for association between ARI diagnosis and isolation of any organism while controlling for potential confounders and accounting for within-patient clustering. A multinomial logistic regression equation assessed for association with specific species. Test characteristics were calculated using bARI diagnosis as the reference standard. **Results**: Among 3,578 respiratory cultures from 533 children (median 4 cultures/child, IQR: 1-9), 25.9% were obtained during ARI and 17.2% had [?]1 organism. Children with ARI diagnosis had higher odds of organism identification (aOR 1.29, 95% CI 1.16–1.44). When controlling for covariates, ARI was associated with isolation of *H. influenzae, M. catarrhalis, S. pneumoniae,* and *S. pyogenes*. Test characteristics revealed a 24.3% sensitivity, 85.2% specificity, 36.5% positive predictive value, and 76.3% negative predictive value in screening for bARI. **Conclusion**: The utility of respiratory culture testing to screen for, diagnose, and direct treatment of ARI in children with tracheostomies is limited.

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This article includes original research. This study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board as exempt research.

This article does not include images of people .

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Abbreviations : ARI, acute respiratory infection; bARI, bacterial acute respiratory infection; BAL, bronchoalveolar lavage; BPD, bronchopulmonary dysplasia; CLDI, chronic lung disease of infancy; CCC, complex chronic condition; CCHMC, Cincinnati Children's Hospital Medical Center; CI, confidence interval; ICD-9, International Classification of Diseases, Ninth Revision; ICD-10, International Classification of Diseases, Tenth Revision; NPV, negative predictive value; OR, odds ratio, aOR, adjusted odds ratio; PPV, positive predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio; TA, tracheostomy aspirate.

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Dr. Steuart contributed to the conceptualization and design the study, performed data collection, assisted with data analysis and validation, interpreted the data, drafted the initial manuscript, and reviewed and revised the manuscript critically for important intellectual content.

Drs. Thomson and Benscoter conceptualized and designed the study, coordinated and supervised data collection, interpreted the data, and reviewed and revised the manuscript critically for important intellectual content.

Dr. Shah conceptualized and designed the study, interpreted the data, and reviewed and revised the manuscript critically for important intellectual content.

Dr. Russell interpreted the data and reviewed and revised the manuscript critically for important intellectual content.

Drs. Beltran-Ale, Ms. Woolums, and Ms. Xia assisted with data analysis and validation, interpreted the data, and reviewed and revised the manuscript critically for important intellectual content.

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Objective : To determine the association of respiratory culture organism isolation with diagnosis of ARI in children with tracheostomies, and assess test characteristics of respiratory cultures in the diagnosis of bacterial ARI (bARI).

Methods : This single-center, retrospective cohort study included respiratory cultures of children with tracheostomies obtained between 2010-2018. The primary predictor was ARI diagnosis code at the time of culture; the primary outcomes were respiratory culture organism isolation and species identified. Generalized estimating equations were used to assess for association between ARI diagnosis and isolation of any organism while controlling for potential confounders and accounting for within-patient clustering. A multinomial logistic regression equation assessed for association with specific species. Test characteristics were calculated using bARI diagnosis as the reference standard.

Results : Among 3,578 respiratory cultures from 533 children (median 4 cultures/child, IQR: 1-9), 25.9% were obtained during ARI and 17.2% had [?]1 organism. Children with ARI diagnosis had higher odds of organism identification (aOR 1.29, 95% CI 1.16–1.44). When controlling for covariates, ARI was associated with isolation of *H. influenzae, M. catarrhalis, S. pneumoniae,* and *S. pyogenes* . Test characteristics revealed a 24.3% sensitivity, 85.2% specificity, 36.5% positive predictive value, and 76.3% negative predictive value in screening for bARI.

Conclusion : The utility of respiratory culture testing to screen for, diagnose, and direct treatment of ARI in children with tracheostomies is limited.

INTRODUCTION

Acute respiratory infections (ARI; e.g. pneumonia, tracheitis) are the most common cause of hospitalization, readmission, and death for children with tracheostomies.[1-3] Clinicians frequently obtain bacterial respiratory cultures in this high risk population, both during times of respiratory illness and when well (i.e., "surveillance"). Depending on the clinical situation, cultures may be used as screening tests, diagnostic tests, or therapy-directing tests. During illness, culture results inform both diagnosis of infection and antibiotic prescribing. Surveillance cultures, when performed, are used to inform the diagnosis and treatment of bacterial colonization of the respiratory tract that may contribute to chronic airway inflammation, increased propensity to recurrent infection, and long-term respiratory decline.[4] However no clinical guideline exists to guide clinician ordering or interpretation of respiratory cultures.[5,6]

Although easy to order and obtain in children with tracheostomies, the interpretation of respiratory cultures is highly complex due to many confounding factors, which have largely been understudied.[7] A major contributor to the difficulty in interpreting culture results in this population is a lack of a robust epidemiologic understanding of organisms expected to be isolated during true ARI, let along during states of wellness. The respiratory tract, unlike other body compartments from which cultures are obtained (e.g., urine, blood), is not a sterile site and harbors oropharyngeal flora in children with and without tracheostomies.[8] Respiratory cultures are often positive when children are not acutely ill among children with and without tracheostomies, although the organisms expected during wellness and their significance is unclear. Furthermore, evolving respiratory microbiome research among children with tracheostomies suggests dynamic bacterial changes during ARI which may change culture interpretation based on illness day.[9,10] The interpretation of respiratory cultures is further obscured by concerns for sampling bias, repeated respiratory culture testing, potential bacterial colonization of the trachea and tracheostomy tube, and laboratory variation.

There is a limited understanding of how respiratory culture growth differs between children with tracheostomies when ill (with ARI) and when healthy (without ARI), limiting the understanding of this test's utility in screening for, diagnosing, and/or treating ARI.[11] Furthermore, although frequently ordered, diagnostic yield and test characteristics of respiratory cultures are unknown. This leads to challenges in diagnosing ARI, deciding when antibiotic therapy is indicated and, when treating, which bacteria to target.

In this study of children with tracheostomies, we sought to determine the epidemiology of respiratory culture organism isolation and to associate organism isolation with clinician-diagnosed ARI. We additionally assessed the performance of respiratory culture in the diagnosis of ARI. We hypothesized that children would have higher likelihood of organism isolation during ARI, and that respiratory cultures have limited predictive utility in screening for and diagnosing ARI.

METHODS

Study Design, Population, and Data Source

This single-center, retrospective cohort study included aerobic bacterial respiratory cultures (tracheostomy aspirate[TA] or bronchoalveolar lavage[BAL]) obtained from children ages 2 months-18 years with tracheostomies at Cincinnati Children's Hospital Medical Center (CCHMC) between January 2010 and December 2018. Inpatient, outpatient, and laboratory-collected cultures were included across the care continuum. Children were identified using an existing internal tracheostomy patient registry maintained by the Division of Pulmonary Medicine. Children with cystic fibrosis were excluded. Detailed demographic, clinical, diagnostic, and respiratory culture data for all encounters were obtained from the electronic medical record (EMR). Culture data was extracted from the EMR

Primary Predictor and Outcome Measures

The primary predictor was diagnosis of any type of ARI at the time of respiratory culture collection, as defined using *International Classification of Diseases, Ninth Revision* (ICD-9) and *Tenth Revision* (ICD-10) diagnostic codes placed by clinicians during the encounter in which the respiratory culture was obtained. Diagnostic codes consistent with conservatively-defined acute bacterial, viral, or nonspecific infection of the trachea or lower respiratory tract (e.g., pneumonia, tracheitis, ventilator-associated pneumonia; **Appendix Table 1**) were identified from review of the Clinical Classification Software-Respiratory Group diagnoses (Agency for Healthcare Research and Quality, Rockville, MD) and selected by group consensus between authors. A secondary predictor was evaluated for the subgroup of children with bacterial-specific ARI diagnoses (bARI) at the time of respiratory culture collection. bARI was defined using previously-identified ICD-9 codes[12] and corresponding ICD-10 codes (e.g., bacterial pneumonia, acute bronchitis due to *Streptococcus* , acute tracheitis; **Appendix Table 2**).

The primary outcome was respiratory culture organism isolation (any isolation and specific organism isolation) in the first respiratory culture obtained in each encounter. Cultures with no speciated organisms or identification of only "oropharyngeal flora" were categorized as "negative". The CCHMC Microbiology Laboratory performs semi-quantification of species for TA cultures and full quantification for BAL cultures. The Microbiology Lab does not have specimen rejection criteria. The Lab defines oropharyngeal flora broadly, and categorizes such species as *Haemophilus* , *S. pneumoniae* , and *M. catarrhalis* as oropharyngeal flora when isolated in small numbers in the presence of other oropharyngeal flora (**Appendix Figure 1**).

Culture Exclusion Criteria

Subsequent respiratory cultures obtained during the same encounter were excluded. For cultures sent from encounters without diagnosis data, encounters were categorized as "no ARI diagnosis" if specimen was a BAL culture sent from the bronchoscopy suite during same-day surgery encounter, as these were presumed to be surveillance cultures consistent with our hospital's clinical practice. All other cultures obtained without encounter diagnoses were excluded.

Covariates

Demographic and patient characteristics that might influence respiratory culture results or interpretation were collected from each encounter, including age and insurance status, location of culture collection (e.g., intensive care unit, acute care floor, office visit, emergency department, laboratory collection, bronchoscopy suite), and hospital department sending culture (e.g., Critical Care, Pulmonary Medicine). At this institution, children with baseline ventilator use do not require ICU admission unless they are clinically unstable.

To characterize clinical complexity and comorbid lung conditions, encounter-level diagnoses were pooled across each child's repeated encounters. Diagnoses were coded to identify complex chronic conditions (CCC) using previously defined codes,[13,14] and bronchopulmonary dysplasia (BPD, *ICD* -9 770.7, *ICD* -10 P27.1) or chronic lung disease of infancy (CLDI, *ICD* -10 P27.8).

Children with chronic *P. aeruginosa* isolation were additionally identified, defined using an adaption to the previously-described criteria in the tracheostomy population[15] - *P. aeruginosa* isolation on [?]50% of cultures obtained in a 12 month period. Gram stain WBC semi-quantification, as categorized by the microbiology laboratory, was additionally examined.

Statistical Analysis

Continuous variables were described using medians and interquartile ranges (IQR). Categorical variables were described using counts and percentages. Patient characteristics and outcomes were stratified by primary exposure and compared using Chi-square or Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables.

To examine the independent association between diagnosis of ARI or bARI and isolation of any organism on respiratory cultures, as well as specific isolation of *P. aeruginosa* and *S. aureus*isolation (the two most frequently isolated organisms), generalized estimating equations were used including potential confounders while accounting for within-patient clustering. Patient demographics included age at culture collection, time with tracheostomy at culture collection, race, ethnicity, and insurance type; measures of clinical complexity included number of complex chronic conditions, BPD or CLDI diagnoses, and baseline ventilator use; clinical variables included respiratory culture source, as well as location and department sending specimen.

To examine the independent association between diagnosis of ARI and all specific organism isolations simultaneously, a multinomial logistic regression equation was calculated including the above potential confounders, accounting for within-patient clustering, and additionally accounting for culture-level clustering among polymicrobial culture results.

To evaluate the test characteristics of the respiratory culture, sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were calculated using clinician-coded diagnosis of bARI as the reference standard and indicator of true bacterial disease. Test characteristics were calculated for the respiratory culture overall and by specimen type (i.e., TA, BAL).

Secondary analyses were conducted examining results of each respiratory culture source (TA and BAL) individually. As isolation of *P. aeruginosa* in both predictor groups may signal these children have chronic colonization, subgroup analysis was conducted examining children with chronic *P. aeruginosa* isolation. Additional subgroup analysis excluding presumed surveillance BAL cultures without diagnosis data was also conducted.

All analyses were performed with R v4.1.1 (Vienna, Austria).[16] P -values <0.05 were considered statistically significant. This study was approved by the CCHMC Institutional Review Board.

RESULTS

Study Cohort

A total of 5,322 bacterial respiratory cultures were obtained among 533 children with tracheostomies across 3,578 inpatient and outpatient encounters during the 9-year study period. After excluding subsequent cultures obtained in the same encounter, 3,578 cultures remained in the final cohort (median 4 cultures per

child, IQR: 1-9, full range 1-45). Approximately one quarter of cultures (25.9%, 926 cultures) were obtained during an encounter with ARI diagnosis (**Table 1**).

Demographic and Clinical Characteristics

Children had a median age at culture collection of 2.8 years (IQR: 1.4-5.5, **Table 1**). Children were medically complex, with 30.7% having 7 or more CCCs. One quarter (26.1%) of children had comorbid BPD/CLDI. Two-thirds of cultures obtained (69.5%) were for children with current or previous chronic home ventilator use. Most cultures were obtained via sterile tracheal aspirate technique (61.4%) and collected during a hospital encounter (83.1%).

Cultures obtained with an accompanying ARI diagnosis were more frequent for children who were younger (2.3 years vs. 2.9 years, p<0.001), publicly insured (60.7% vs 53.5%, p<0.001), and without a diagnosis of BPD/CLDI (23.7% vs 27.0%, p<0.001) as compared with children with no ARI diagnosis (**Table 1**). Cultures obtained during ARI were also more frequent among children with 7+ CCCs (38.3% vs 28.0%, p<0.001). Differences in encounter and department at collection were observed, with cultures collected during ARI being less frequently obtained during an office visit, laboratory visit (i.e., specimen collection only visit), and in the bronchoscopy suite or post-anesthesia care unit (PACU). Cultures collected during ARI were much more frequently TA specimens (83.9%) versus BAL specimens, while cultures collected without ARI were only slightly more frequently TA specimens (53.6%, p<0.001, **Table 1**).

Most (77%) encounters with ARI diagnosis also met bARI diagnosis criteria (**Table 1**). Cultures obtained in children with bARI diagnosis were similar to those with ARI diagnosis, except that BPD/CLDI diagnosis was less frequent among with bARI (21.7%), PACU location was less frequent (1.6%), and TA specimen type was more frequent (90.2%,**Table 1**).

Organism Isolation Outcomes

Among all cultures obtained, only 617 cultures (17.2%) demonstrated organism isolation, with 751 total organisms identified (22 unique species). Unadjusted analysis demonstrated that cultures of children with ARI had higher odds of any organism isolation compared with those from children without ARI (24.3% vs 14.8%, p<0.01,**Figure 1(a)**); unadjusted odds ratio(OR) 1.83 (95% Confidence Interval(CI) 1.67-2.01, **Table 2**). The most prevalent organisms isolated in both children with and without ARI were *Pseudomonas aeruginosa* , *Staphylococcus aureus* (29% methicillin-resistant),*Haemophilus influenzae* , and *Moraxella catarrhalis*(**Figure 1(b)**). On unadjusted analysis, all 4 of these organisms were significantly more frequently isolated with ARI diagnosis compared with no ARI diagnosis (**Figure 1(b)**).

On adjusted analysis, ARI diagnosis continued to demonstrate increased odds of organism isolation (aOR 1.29; 95%CI 1.16-1.44, **Table 2**). ARI was not associated with *P. aeruginosa* or *S. aureus* isolation on adjusted analysis, but *P. aeruginosa* was associated with bARI diagnosis. Using multinomial adjusted analysis examining for the comprehensive outcome of each organism isolation, ARI diagnosis was associated with isolation of 4 organisms, *H. influenzae* (aOR 2.09), *M. catarrhalis* (aOR 1.76), *S. pyogenes* (aOR 1.92), and *Streptococcus pneumoniae* (aOR 4.04,**Table 3**), as compared with no organism isolation. Isolation of all other organisms, including *P. aeruginosa* and *S. aureus* , were not associated with ARI diagnosis at the time of culture collection.

Test Characteristics

Respiratory culture testing demonstrated low sensitivity (24.3%) and moderate specificity (85.2%) as screening tests for ARI in children with tracheostomies (**Table 4**), indicative of poor test concordance with clinician diagnosis. Similarly, respiratory cultures demonstrated a poor positive predictive value (36.5%) and only a fair negative predictive value (76.3%), indicating limited effectiveness at identifying ARI in children with tracheostomies. Positive likelihood ratio(+LR) was determined to be 1.64, and negative likelihood ratio(-LR) 0.89. BAL specimens had a higher specificity (90.4%) and negative predictive value (90.1%), but still generally poor test characteristics. TA cultures had very poor test performance as screening tests in all measures (**Table 4**).

Secondary Analyses

In secondary analysis examining respiratory cultures by specimen source, ARI diagnosis remained associated with overall organism isolation among both TA cultures and BAL cultures (**Table 5(a)**). ARI remained not associated with *P. aeruginosa* or *S. aureus* isolation within both specimen source types. In subgroup analysis examining children with chronic *P. aeruginosa* isolation (115 cultures among 20 children), children had similar distribution of the most common organism species identified with and without ARI, with the exception of*M. catarrhalis* (**Appendix Figure 2**). ARI remained associated with overall organism isolation in this subgroup of children with chronic *P. aeruginosa* (**Table 5(b)**). In subgroup analysis excluding 417 cultures (11.7% of the total cohort) obtained from same-day bronchoscopy encounters with no diagnosis data (presumed surveillance cultures), ARI diagnosis remained associated with organism isolation (**Table 5(c)**).

DISCUSSION

In this retrospective cohort study of all respiratory cultures from children with tracheostomies, cultures obtained in children with a diagnosis of ARI had a 29% higher odds of overall organism isolation compared to children without ARI. Although many organism species were isolated in both groups, ARI diagnosis was only associated with isolation of 4 specific organisms (*H. influenzae* , *M. catarrhalis* , *S. pyogenes* , and *S. pneumoniae)* , all of which are expected in usual bacterial respiratory infections. After controlling for patient-level factors and serial culture sampling, isolation of *P. aeruginosa* was not associated with ARI diagnosis, suggesting this organism may not be considered causative of ARI and may not require acute treatment. Furthermore, respiratory cultures performed poorly as a screening test for bARI, with low sensitivity and moderate specificity. Taken together, our data suggest that the utility of respiratory culture testing to diagnose, direct treatment for, or even screen for ARI and bARI among children with tracheostomies is limited.

The clinical interpretation of respiratory cultures in children with tracheostomies is highly complex, both during states with ARI and without ARI, and is becoming more nuanced as epidemiology evolves.[7] The respiratory tract is not a sterile site and many factors influence the dynamic composition of oropharyngeal flora in children with and without artificial airways.[8,17,18] Chronic bacterial colonization of the respiratory tract is a presumed entity, but is difficult to define and identify in practice.[19-21] Emerging studies are illuminating the complex interaction of the respiratory tract flora with viruses (i.e., *Haemophilus* and *Moraxella* "blooms"), colonizing bacteria, inflammatory biomarkers, and the microbiome-host interplay.[10,22-26] Thus the traditional, reductionist notion of attributing an ARI to a single bacteria, virus, or even single group of pathogens is increasingly recognized as inadequate.[9,27]

In our study, respiratory cultures obtained during ARI had higher adjusted odds of identifying a bacterial organism, a finding which seems to support some role of respiratory culture testing in diagnosing ARI. Presumably, the identified organism is a pathogenic cause of ARI in many of these circumstances, though the nuanced reasons stated above and retrospective studies in related populations would argue the isolation of some organisms may still be circumstantial.[11,19,28]Despite this seemingly straightforward association, this study highlights other perplexing and contradictory epidemiologic culture findings. One the one hand, the majority of respiratory cultures in children with ARI (76.9%) in our study were negative; such a finding could be the result of multiple factors including an underlying viral etiology of ARI in many of these children (who would not be expected to have positive bacterial cultures), symptomatic microbiome shifts[27,29] or bacterial "blooms",[10] inadequate culture sampling, or a generally low yield of the respiratory culture test. In contrast, a number of positive respiratory cultures were identified among children without ARI; in the absence of a clinician diagnosis of ARI, positive cultures may indicate clinician interpretation of chronic bacterial colonization of the respiratory tract.[19,20,30,31]These contradictory culture findings suggest that respiratory culture positivity alone has unclear diagnostic yield in differentiating acute bacterial infection, viral infection, and chronic colonization.

During ARI in children with tracheostomies, clinicians may use respiratory culture results to inform antibiotic prescribing decisions including continuation, modification, or discontinuation of therapy. Increased respira-

tory culture acquisition has been associated with increased antibiotic use among children's hospitals.[32,33] In our study only 4 species remained associated with ARI on adjusted, multinomial analysis. These species, *H. influenzae* , *M. catarrhalis* , *S. pyogenes* , and *S. pneumoniae* , are all expected organisms implicated in usual bacterial respiratory infections among other populations.[34] Evidence-based treatment guidelines typically recommend empiric antibiotic therapy directed at these pathogens in uncomplicated cases, without need for organism-identification testing. The association of ARI with this group of organisms challenges the utility of respiratory cultures in directing antibiotic treatment among children with tracheostomies.

Adjusted analysis demonstrated that ARI diagnosis was not associated with isolation of *P. aeruginosa* and *S. aureus* . Isolation of these two species is thus more likely related to patient factors (e.g., ventilator dependence, time with tracheostomy tube), and recurrent sampling, as opposed to true ARI. With an equal likelihood of identification both with and without ARI, our findings imply that these organisms may not be causative of ARI but instead be consistent with chronic colonization. Given the weak association of *P. aeruginosa*identification with bARI diagnosis, there may be some clinician interpretation of this organism as additionally disease-causing in certain circumstances. Both *P. aeruginosa* and *S. aureus*are known to cause biofilm formation of airway devices, a fact which may or may not be the same as airway colonization.[30,31,35,36] This study questions the necessity for reflexive anti-pseudomonal and anti-staphylococcal antibiotic treatment of positive cultures in some clinical situations, such as in the absence of infectious symptoms or when viral mono-infection is suspected. How these potentially colonizing airway bacteria may contribute to ARI susceptibility and frequency is not known.[27]

As a screening test for bARI, the respiratory culture overall demonstrated unfavorable test characteristics.[37]Respiratory cultures correctly identified only 27.2% of children diagnosed by clinicians with bARI (sensitivity), and had only a 32.9% probability of identifying children with bARI (PPV) with a high false positive rate. The culture had a somewhat better probability of identifying children without bARI (85.4% specificity, 81.7% NPV).[37] In this case in which both false positive and false negative test results are common, and both are associated with measurable harm including antibiotic overuse, these test statistics are unsatisfactory. For the clinician at bedside, the positive and negative likelihood ratios for respiratory cultures here are modest at best, and suggest respiratory cultures are not particularly clinically useful as bARI screening tests. This is corroborated by recent evidence of poor concordance between tracheal aspirate and BAL cultures.[38]

Further evaluation of treatment approaches and clinical outcomes for both positive and negative cultures in relation to ARI diagnosis are needed. In the absence of clinical guidelines to direct clinician ordering or interpretation of respiratory cultures, clinicians order and interpret respiratory cultures in different ways.[5,11]Clinicians may interpret respiratory culture results in the context of the individual patient, the clinician's experience, institutional norms, and knowledge of local microbiology lab practices. However, studies have demonstrated wide inter-institutional variability in culture ordering practices, microbiology lab processing, culture results (both positivity rate and organism type), and clinician response to results.[5,7,39] Variability in sample quality and lab processing may also contribute, as has been documented in endotracheal tube cultures.[39] The consequence of this variability is clinical inconsistency in diagnosing ARI,[5] use of and types of antibiotic treatment,[32] and probably also patient outcomes including severity, length of illness, and respiratory support needs, all of which limit research and improvement efforts.

This study has several limitations. The use of diagnostic codes to define ARI and bARI predictor status could lead to misclassification of predictor for children with and without these conditions. Although we restricted codes to those most consistent with true infection, it is possible that cultures were incorrectly classified. Furthermore, the association of positive bacterial cultures with ARI cannot imply bacterial causation of these infections; our retrospective study was not designed to explore causation of respiratory bacteria in ARI. The retrospective design of this study also creates the potential for residual confounding; there may be other clinical or demographic factors influencing respiratory culture acquisition and interpretation that we are unable to capture with our discrete dataset. Furthermore, our center's results may not be generalizable to children with tracheostomies at other institutions. Our institution's positive culture prevalence is lower than

that observed at other institutions;[15,40] this is consistent with prior internal studies, and is hypothesized to be related to differences in our population and/or local factors (e.g. infection control policies, lab reporting procedures).

CONCLUSION

Among children with tracheostomies at our institution, respiratory culture testing demonstrates limited utility as a testing tool to screen for, diagnose, or treat, ARI. ARI diagnosis is only associated with isolation of organisms commonly seen in and empirically treated during routine bacterial respiratory infections, without better odds of identifying uncommon or treatment-modifying organisms. Despite frequent isolation, *P. aeruginosa* was not associated with ARI and may not represent acute infection. Both TA and BAL respiratory cultures have poor sensitivity, moderate specificity, and low likelihood ratios to screen for ARI in clinical practice. Future guidelines for respiratory culture ordering and clinical interpretation should consider the limited value of this diagnostic test.

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Table 1. Respiratory culture cohort clinical characteristics.

	Full cohort (n = 3,571)	**Full cohort (n = 3,571)**	**With ARI (n = 968)**	**With ARI (n = 968)**	**No ARI (n= 2,603)**	**No ARI (n= 2,603)**	**No ARI (n= 2,603)**	**p-value[a]**	**p-value[a]**	**p-value[a]**	**p-value[a]**	**W h (7**
Demographics	(%)	**n**	(%)	**n**	(%)	(%)						**n**

	Full cohort (n = 3,571)	**Full cohort (n = 3,571)**	**With ARI (n = 968)**	**With ARI (n = 968)**	**No ARI (n= 2,603)**	**No ARI (n= 2,603)**	**No ARI (n= 2,603)**	**p-value[a]**	**p-value[a]**	**p-value[a]**	**p-value[a]**	**W h (7**
Age in years (median, [IQR])	2.8	[1.4, 5.5]	2.3	[1.1, 4.7]	2.9	[1.6, 5.8]	[1.6, 5.8]	<0.001	<0.001	<0.001	<0.001	2
Male gender	1,932	(54.1%)	546	(56.4%)	1,386	(53.2%)	(53.2%)	0.10	0.10	0.10	0.10	4
Publically insured	1,980	(55.4%)	562	(60.7%)	1,418	(53.5%)	(53.5%)	<0.001	<0.001	<0.001	<0.001	4
Race	2,558	(71.6%)	689	(71.2%)	1,869	(71.8%)	(71.8%)	0.86	0.86	0.86	0.86	5
White	638	(17.9%)	173	(17.9%)	465	(17.9%)	(17.9%)					1
Black	375	(10.5%)	106	(11.0%)	269	(10.3%)	(10.3%)					
Other												
Hispanic ethnicity	145	(4.1%)	46	(4.8%)	99	(3.8%)	(3.8%)	0.24	0.24	0.24	0.24	3
Clinical Characteristics												
BPD/CLD diagnosis[b]	932	(26.1%)	233	(24.1%)	699	(26.9%)	(26.9%)	<0.001	<0.001	<0.001	<0.001	1
Number of complex chronic conditions	273	(7.6%)	47	(4.9%)	226	(8.7%)	(8.7%)	<0.001	<0.001	<0.001	<0.001	3
1-2	726	(20.3%)	145	(15.0%)	581	(22.3%)	(22.3%)					3
3-4	1,476	(41.3%)	406	(41.9%)	1,070	(41.1%)	(41.1%)					2
5-6	879	(24.6%)	295	(30.5%)	584	(22.5%)	(22.5%)					
7-8	217	(6.1%)	75	(7.7%)	142	(5.5%)	(5.5%)					
9-12												
Time of tracheostomy in years (median, [IQR])	1.8	[0.8, 3.5]	1.4	[0.4, 3.1]	1.9	[0.9, 3.6]	[0.9, 3.6]	<0.001	<0.001	<0.001	<0.001	1

	Full cohort (n = 3,571)	**Full cohort (n = 3,571)**	**With ARI (n = 968)**	**With ARI (n = 968)**	**No ARI (n= 2,603)**	**No ARI (n= 2,603)**	**No ARI (n= 2,603)**	**p-value[a]**	**p-value[a]**	**p-value[a]**	**p-value[a]**	**V b (** **7**
Baseline ventilator use[c]	2,484	(69.6%)	675	(69.7%)	1,809	(69.5%)	(69.5%)	0.92	0.92	0.92	0.92	5
Encounter type at culture collection	2,966	(83.1%)	867	79 (89.6%)	2,099	(80.6%)	(80.6%)	<0.001	<0.001	<0.001	<0.001	6
Hospital or ED	368	(10.3%)	22	(8.2%)	289	(11.1%)	(11.1%)					1
Office visit	237	(6.6%)		(2.3%)	215	(8.3%)	(8.3%)					
Specimen collection only												
Department at culture collection	1,394	(39.0%)	638	(65.9%)	756	(32.8%)	(32.8%)	<0.001	<0.001	<0.001	<0.001	5
Acute care	251	(7.0%)	112	64 (11.6%)	139	(5.3%)	(5.3%)					1
Critical care	999	(28.0%)	49	55 (6.6%)	935	(35.9%)	(35.9%)					5
OR/Bronchoscopy suite	289	(8.1%)	50	(5.1%)	240	(9.2%)	(9.2%)					
Emergency Department	244	(6.8%)		(5.7%)	189	(7.3%)	(7.3%)					
Pulmonology, ENT Clinic	394	(11.0%)		(5.2%)	344	(13.2%)	(13.2%)					
Other Clinic[d] or Lab												

	Full cohort (n = 3,571)	**Full cohort (n = 3,571)**	**With ARI (n = 968)**	**With ARI (n = 968)**	**No ARI (n= 2,603)**	**No ARI (n= 2,603)**	**No ARI (n= 2,603)**	**p-value[a]**	**p-value[a]**	**p-value[a]**	**p-value[a]**	**W b (7**
Respiratory Culture Characteristics	**Respiratory Culture Characteristics**											
Specimen source	2,176	(60.9%)	811	(83.8%)	1,365	(52.4%)	(52.4%)	<0.001	<0.001	<0.001	<0.001	6
Tracheostomy aspirate	1,395	(39.1%)	157	(16.2%)	1,238	(47.6%)	(47.6%)					
Bronchoalveolar lavage												
Polymicrobial growth	134	(3.8%)	48	(5.0%)	86	(3.3%)	(3.3%)	0.03	0.03	0.03	0.03	4
WBCs quantified on gram stain[e]	1,414	(39.6%)	479	(49.5%)	935	(35.9%)	(35.9%)	<0.001	<0.001	<0.001	<0.001	3
Many	731	(20.5%)	189	(19.5%)	542	(20.8%)	(20.8%)					1
Moderate	723	(20.2%)	177	82 (18.3%)	546	(21.0%)	(21.0%)					1
Few	458	(12.8%)	33	(8.5%)	376	(14.4%)	(14.4%)					2
Very few	204	(5.7%)		(3.4%)	171	(6.6%)	(6.6%)					
None												

Abbreviations: IQR: interquartile range; OR: operating room; WBCs: white blood cells.

[a] p-value was determined using Chi-square or Fisher's exact tests for categorical variables and Wilcoxon rank sum tests for continuous variables.

[b] Excludes 264 cultures for which no BPD diagnosis information is available.

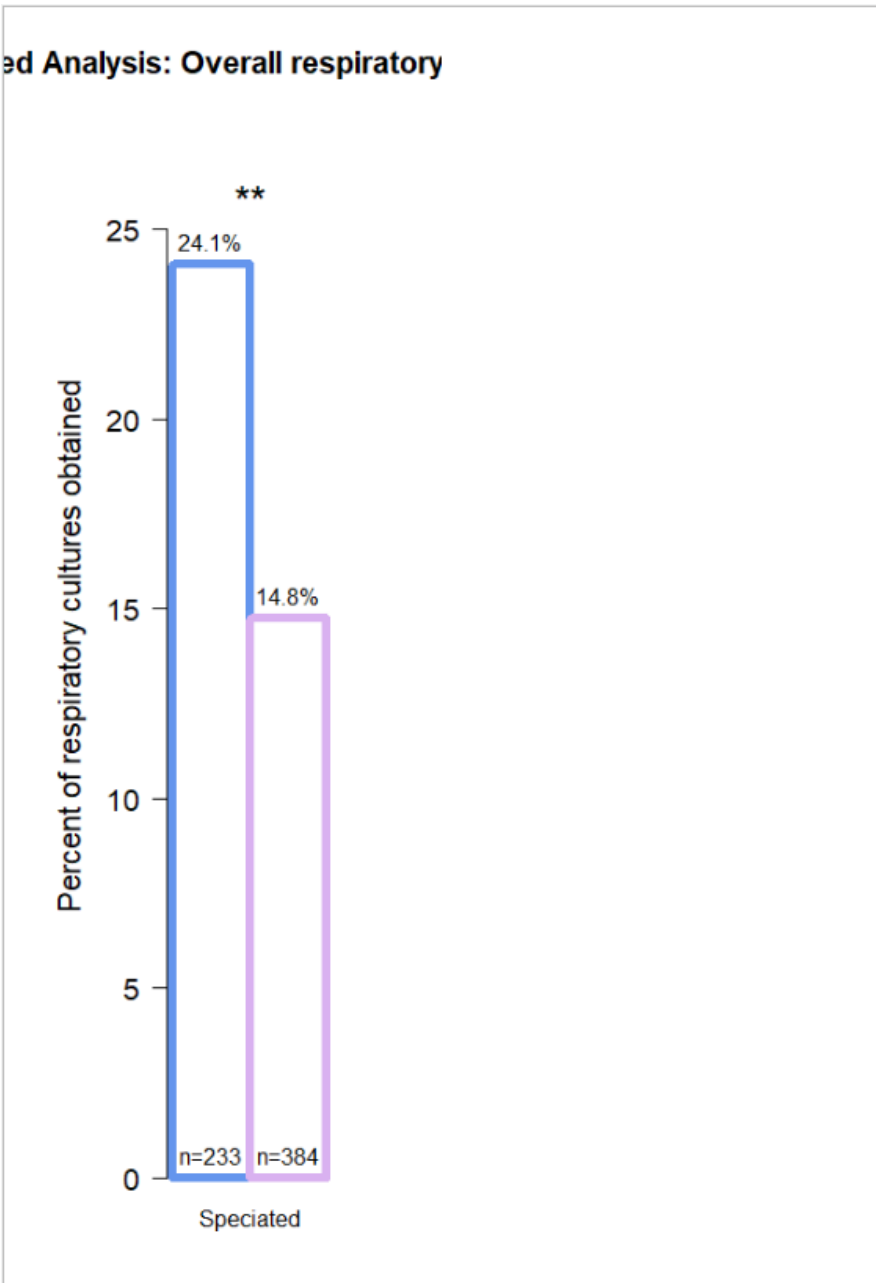
[c] Current or previous baseline ventilator use.

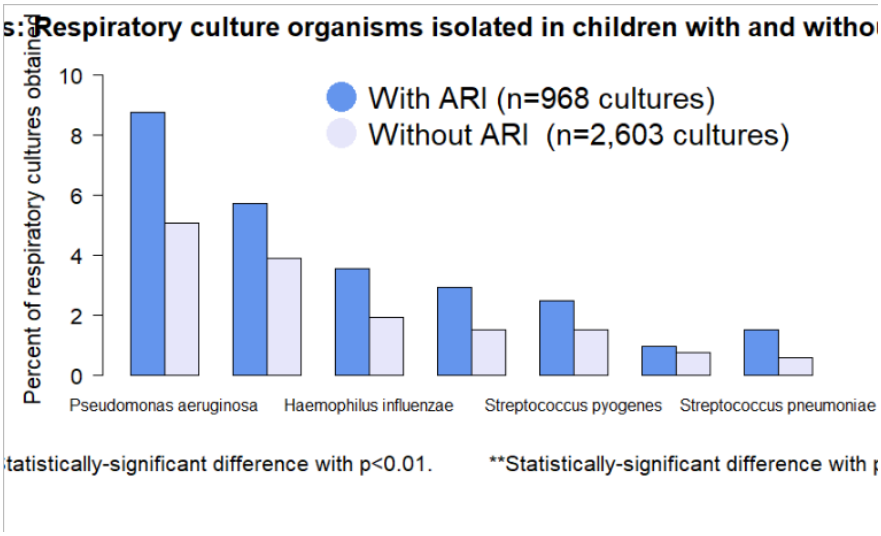
[d] Includes primary care and subspecialty clinics.

[e] Excludes 41 culture results without documentation of WBC count.

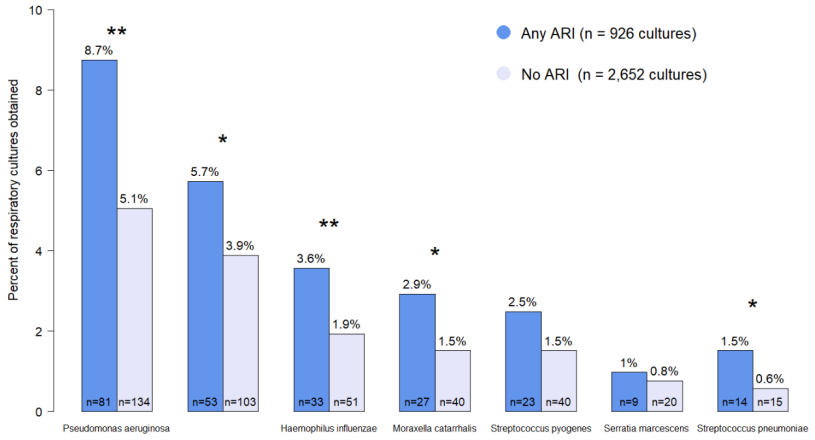
Figure 1. Unadjusted Analysis of Respiratory Culture[a] Results among children with and without acute respiratory infection (ARI). (a) Overall culture speciation. (b) Organism-specific speciation.

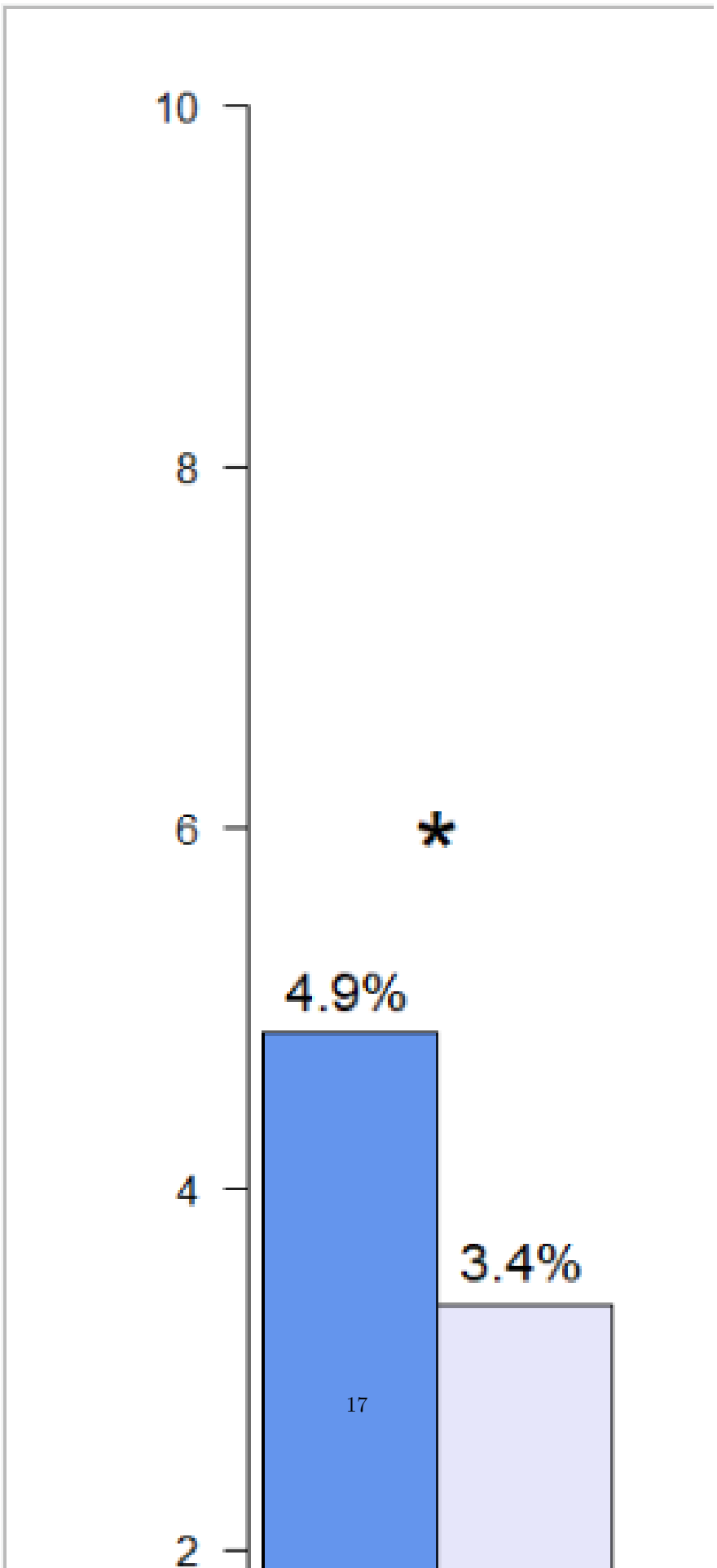
(c) Speciation among children with bacterial acute respiratory infection (bARI).





e 1b. Unadjusted Analysis: Respiratory culture organisms isolated in children with and without acute respiratory infectio





For bARI (n=745)	27.2%	9.9%	6.3%	3.4%	3.4%	2.7%	1.1%	1.6%	6.2%
	n = 203	74	47	25	25	20	8	12	46

[a] Respiratory cultures sources: tracheostomy aspirate or bronchoalveolar lavage.

[b] 29% of *S. aureus* isolated demonstrated methicillin-resistance.

*Statistically-significant difference with p<0.05, **p<0.01.

Table 2. Unadjusted and Adjusted Analyses: Respiratory culture organism isolation during ARI and bARI.

Any ARI	**Unadjusted OR[a]**	**Unadjusted OR[a]**	**Unadjus**
Any organism isolation[c]	1.83	1.83	1.83
Pseudomonas aeruginosa isolation[d]	*Pseudomonas aeruginosa* isolation[d]	*Pseudomonas aeruginosa* isolation[d]	1.68
Staphylococcus aureus isolation[e]	*Staphylococcus aureus* isolation[e]	1.39	1.39
bARI	**Unadjusted OR[a]**	**Unadjusted OR[a]**	**Unadjus**
Any organism isolation[c]	2.18	2.18	2.18
Pseudomonas aeruginosa isolation[d]	2.10	2.10	2.10
Staphylococcus aureus isolation[e]	1.67	1.67	1.67

Abbreviations: ARI: acute respiratory illness; OR: odds ratio; CI: confidence interval.

[a] Calculated using a generalized estimating equation accounting for within-patient clustering.

[b] Calculated using a generalized estimating equation to control for patient-level covariates [including: number of complex chronic conditions, ventilator use, race, ethnicity, insurance type], culture-level covariates [including: age at culture acquisition, time with tracheostomy at culture acquisition, culture source (tracheostomy aspirate or bronchoalveolar lavage), department obtained], and to account for within-patient clustering.

[c] versus no organism isolation.

[d] versus no *P. aeruginosa* isolation.

[e] versus no *S. aureus* isolation.

Table 3. Adjusted analysis of organism-specific isolation during ARI.

Organism	**RRR[a]**	**95% CI**	**p-value**
No growth/Oropharyngeal flora	(reference)	(reference)	(reference)
Burkholderia species	0.014	0.001 – 2.61	0.41
Corynebacterium species	1.13	0.65 – 1.96	0.82
Enterobacter species	0.92	0.48 – 1.77	0.90
Escherichia coli	0.30	0.09 – 1.05	0.34
Haemophilus influenza	**2.09**	**1.60 – 2.73**	**0.006**
Klebsiella pneumoniae	0.78	0.42 – 1.45	0.69
Moraxella catarrhalis	**1.76**	**1.32 – 2.33**	**0.04**
Pseudomonas aeruginosa	1.08	0.92 – 1.27	0.63
Serratia marcescens	1.07	0.69 – 1.68	0.88
Staphylococcus aureus	0.95	0.77 – 1.15	0.77
Stenotrophomonas maltophilia	0.92	0.54 – 1.56	0.87
Streptococcus pneumoniae	**4.04**	**2.61 – 6.27**	**0.001**

Organism	RRR[a]	95% CI	p-value
Streptococcus pyogenes Yeast Other organism[b]	**1.92** 0.42 0.67	**1.44 – 2.64** 0.14 – 1.28 0.44 – 1.04	**0.03** 0.43 0.36

Abbreviations: ARI: acute respiratory illness; RRR: relative risk ratio; OR: odds ratio; CI: confidence interval.

[a] Calculated using a multinomial logistic regression equation to control for patient-level covariates [including: number of complex chronic conditions, ventilator use, race, ethnicity, insurance type], culture-level covariates [including: age at culture acquisition, time with tracheostomy at culture acquisition, culture source (tracheostomy aspirate or bronchoalveolar lavage), department obtained], and to account for within-patient clustering and, for polymicrobial cultures, within-culture clustering.

[b] Other organism category includes*Achromobacter, Chryseobacterium, Citrobacter, Elizabethkingia, Enterococcus, Delftia, Flavobacterium, Neisseria, Nocardia, Pantoea, Proteus, and Raoultella* species, and *Streptococcus dysgalactiae.*

Table 4: Respiratory Culture Test Characteristics for bARI.

Test characteristic*	Any respiratory culture	Tracheostomy aspirate (TA) culture	Bronchoalveolar lavage (BAL) culture
Sensitivity	27.2%	28.4%	16.4%
Specificity	85.4%	81.8%	89.9%
Predictive Values	32.9%	40.5%	8.3%
Positive Predictive Value			
Negative Predictive Value	81.7 %	71.8%	95.1%
Likelihood Ratios	1.86	1.54	1.62
Positive Likelihood Ratio			
Negative Likelihood Ratio	0.85	0.88	0.93

*Test characteristics calculated using diagnosis of bARI as the reference standard and pathogenic bacterial identification as criteria for a positive test result.

Table 5. Secondary analyses.

By specimen source	Adjusted OR[a]	Adjusted OR[a]	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI	p-value
Tracheostomy aspirate									
Any organism isolation	**1.30**	**1.30**	**1.30**	**1.30**	**1.16 – 1.46**	**1.16 – 1.46**	**1.16 – 1.46**	**0.025**	**0.025**
Pseudomonas aeruginosa isolation	0.99	0.99	0.99	0.99	0.84 – 1.17	0.84 – 1.17	0.84 – 1.17	0.95	0.95

By specimen source	**Adjusted OR[a]**	**Adjusted OR[a]**	**95% CI**	**95% CI**	**95% CI**	**95% CI**	**95% CI**	**95% CI**	**p-value**
Staphylococcus aureus isolation	0.97	0.97	0.97	0.97	0.80 – 1.19	0.80 – 1.19	0.80 – 1.19	0.90	0.90
Bronchoalveolar lavage									
Any organism isolation	**2.11**	**2.11**	**2.11**	**2.11**	**1.65 – 2.68**	**1.65 – 2.68**	**1.65 – 2.68**	**0.002**	**0.002**
Pseudomonas aeruginosa isolation	1.25	1.25	1.25	1.25	0.69 – 2.27	0.69 – 2.27	0.69 – 2.27	0.70	0.70
Staphylococcus aureus isolation	0.99	0.99	0.99	0.99	0.48 – 2.02	0.48 – 2.02	0.48 – 2.02	0.85	0.85
Chronic *P. aeruginosa*[b]	**Adjusted OR[c]**	**Adjusted OR[c]**	**Adjusted OR[c]**	**95% CI**	**95% CI**	**95% CI**	**95% CI**	**p-value**	**p-value**
Any organism isolation	**2.52**	**2.52**	**2.52**	**1.77 – 3.58**	**1.77 – 3.58**	**1.77 – 3.58**	**1.77 – 3.58**	**0.009**	**0.009**
Pseudomonas aeruginosa isolation	0.90	0.90	0.90	0.90	0.90	0.72 – 1.11	0.72 – 1.11	0.62	0.62
Staphylococcus aureus isolation	1.28	1.28	1.28	1.28	1.28	0.15 – 11.1	0.15 – 11.1	0.91	0.91
Excluding encounters with no diagnosis information	**Adjusted OR[a]**	**Adjusted OR[a]**	**Adjusted OR[a]**	**Adjusted OR[a]**	**Adjusted OR[a]**	**95% CI**	**95% CI**	**p-value**	**p-value**
Any organism isolation	**1.28**	**1.28**	**1.28**	**1.28**	**1.28**	**1.15 – 1.42**	**1.15 – 1.42**	**1.15 – 1.42**	**0.018**
Pseudomonas aeruginosa isolation	0.97	0.97	0.97	0.97	0.97	0.83 – 1.13	0.83 – 1.13	0.83 – 1.13	0.84

By specimen source	Adjusted OR[a]	Adjusted OR[a]	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI	p-value
Staphylococcus aureus isolation	0.92	0.92	0.92	0.92	0.92	0.76 – 1.11	0.76 – 1.11	0.76 – 1.11	0.65

Abbreviations: OR: odds ratio; CI: confidence interval.[a] Calculated using a generalized estimating equation to control for patient-level covariates [including: number of complex chronic conditions, ventilator use, race, ethnicity, insurance type], culture-level covariates [including: age at culture acquisition, time with tracheostomy at culture acquisition, department obtained], and to account for within-patient clustering.[b] Examining the 115 cultures of the 20 children who had chronic *P. aeruginosa* isolation, defined as *P. aeruginosa* isolation on [?]50% of respiratory cultures obtained in a 12 month period in the same child, using an adaptation of previously-described criteria.[15c] Calculated using a generalized estimating equation to control for patient-level covariates [including: number of complex chronic conditions, ventilator use, race], culture-level covariates [including: age at culture acquisition, time with tracheostomy at culture acquisition], and to account for within-patient clustering. For *Staphylococcus* analysis, covariates included only number of complex chronic conditions and ventilator use.